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ORIGINAL ARTICLE

Determination of Cefoxitin Sodium in Presence of Its Alkali-induced Degradation Product Using a Univariate Constant Center Spectrophotometric Method and Three Multivariate Chemometric Methods: A Comparative Study

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ABSTRACT

This study aimed to develop four accurate and precise spectrophotometric methods for determination of cefoxitin-sodium in presence of its alkali-induced degradation product. The first one was a univariate spectrophotometric constant center method and the other three multivariate chemometric methods namely; Savitsky-Golay filters, continuous wavelet transform of ratio spectra and wavelet transform of the first derivative of ratio spectra. The accuracy, precision, and linearity ranges of the proposed methods were determined and the four methods were successfully applied for the determination of cefoxitin-sodium in pure form and in its powder for injection. The proposed methods were simple, rapid, economic, and accurate. Comparison between the results obtained with those of a reported method was done, no significant differences between the proposed methods and the reported one.
Keywords: Cefoxitin-sodium; constant center; multivariate chemometric; Savitsky-Golay filters; continuous wavelet transform.

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INTRODUCTION

Most antibiotics used to treat humans are microbial natural products or semisynthetic derivatives of these molecules. Cephamycin C is a natural product with antibiotic activity produced by *Streptomyces lactamdurans*, since that biological product is altered to artificially synthesize it to broadened its spectrum, This new modification produced compound called cefoxitin, which bears the same relationship to the parent compound [1, 2].

Cefoxitin-sodium is a semisynthetic cephamycin antibiotic classified as a second generation cephalosporin chemically named; sodium 3-carbamoyloxymethyl-7-methoxy-7-[2-(2-thienyl) acetamido]-3-cephem-4-carboxylate[1]. (Figure1). An alpha-oriented methoxyl-group at C-7 increased the steric bulk, which conveys very significant stability against β-lactamases [2]. It is used for the treatment of infections caused by anaerobic and mixed aerobic anaerobic infections, it is not absorbed from gastrointestinal tract so it's given parenterally as sodium salt [3,4]. Few HPLC methods were developed for the determination of cefoxitin-sodium [5-9], TLC method [10], LC-MS/MS [11], a flow injection chemiluminescent methods [12], colorimetric determination of cefoxitin-sodium [13-15] and first derivative estimation of cefoxitin-sodium in binary mixture with cephalothin at 236.75 were also reported [16]. Cefoxitin-sodium was stressed with 4.5 M sulphuric acid on boiling water bath for 20 min.,

2nd derivative spectra were recorded at 278 nm [17]. A stability indicating method by spectrofluorimetric analysis [18] was also described for its analysis.

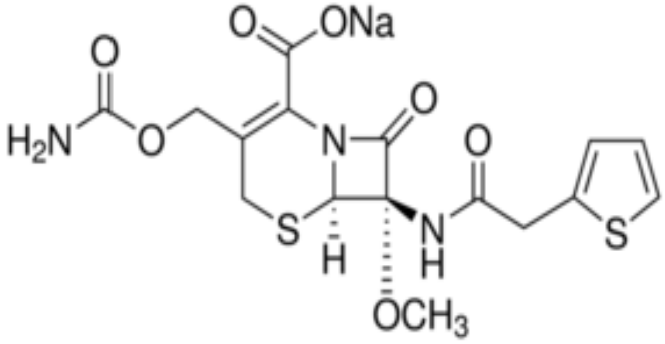

Figure (1): Chemical structure of cefoxitin-sodium.

This work introduced four spectrophotometric methods for determination of cefoxitin-sodium in presence of its alkali-induced degradation product namely; constant center, Savitsky-Golay filters, continuous wavelet transform of ratio spectra and wavelet transform of the first derivative of ratio spectra.

MATERIAL AND METHODS

2. Experimental

2.1. Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and aHP1020 laser jet printer. The spectral band was 2 nm and scanning speed is 2800 nm/min and a 1nm data interval.

2.2. Software:

- Constant center method was done with the bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU).
- Savitsky-Golay filters; was done with our own written code in Matlab 8.2.0.701 (R2013b).
- Continuous wavelet transform and derivative wavelet were done with our own written code in Matlab 8.2.0.701 (R2013b) in conjugation with wavelet toolbox.
- The *t*-test and *F*-test were performed using Microsoft® Excel.

2.3. Chemicals and reagents

- Cefoxitin sodium (98.8%) was kindly supplied by Pharco B International Co., Cairo, Egypt. Lot no.12052036.
- Primafoxin® 1gm vial for IV injection & IV infusion, labeled to contain1gm of cefoxitin sodium per vial, Batch No.(109), the product of Pharco B international Co., Egypt, were purchased from local pharmacies.
- 0.1N Hydrochloric acid, 0.1N Sodium hydroxide (El-Nasr Co., Egypt).
- Methanol (sigma-Aldrich, USA).
- Distilled water.

2.4. Standard solutions for multivariate chemometric methods

- **Standard solution of intact cefoxitin-sodium:**

A stock solution (1mg mL^{-1}) was prepared by dissolving 100 mg of cefoxitin-sodium in few milliliters water, and then the volume was adjusted to 100 mL with water.

Working solution (0.1 mg mL^{-1}) was prepared by suitable dilution from the stock solution using distilled water as solvent.

- **Stock solution of cefoxitin-sodium alkali-induced degradation product [19, 20]:**

The stock solution of cefoxitin-sodium alkali-induced degradation product (1mg mL^{-1}) was prepared by treating 100 mg of cefoxitin-sodium with 30 mL 0.1N NaOH, then was refluxed for 10 min. and cooled to room temperature, neutralized with 0.1N HCl and evaporated to dryness, the residue was dissolved in methanol, filtered, and again evaporated where the residue was dissolved in 60 mL water and completed to 100 mL with distilled water.

Working solution of cefoxitin-sodium alkali-induced degradation product (0.1 mg mL^{-1}) was prepared by suitable dilution from the stock solution using distilled water.

3. Procedure

3.1. Linearity and range:

Different aliquots of the cefoxitin-sodium working solution or from cefoxitin-sodium alkali-induced degradation product (0.1 mg mL^{-1}) ranging from (2-32 µg mL^{-1}) were transferred to 10-mL volumetric flasks and completed to volume with water. Absorption spectra were scanned (from 200 to 400 nm) and stored in the computer using water as a blank.

3.1.1. Univariate spectrophotometric determination of cefoxitin-sodium in presence of its-alkali-induced degradation product using constant center spectrophotometric (CCSM) Method [21-24]

i) Cefoxitin-sodium:

The calibration curve relating the absorbance of the zero order spectra of cefoxitin-sodium at λ_{max} 235 nm versus the corresponding concentrations was constructed, the regression equation was computed.

(ii) Cefoxitin-sodium alkali-induced degradation product:

The stored absorption spectra of degradation product were divided by the absorption spectrum of cefoxitin-sodium (20 µg mL^{-1}) to obtain the ratio spectra. The calibration curve relating the difference between the amplitudes of the obtained ratio spectra at (238 and 275 nm) and amplitudes at 275 nm was constructed, the regression equation was computed.

3.1.2. Multivariate spectrophotometric determination of cefoxitin-sodium in presence of its alkali-induced degradation product using ratio derivative by Savitsky-Golay filters [25-29]**:**

The stored absorption spectra of cefoxitin-sodium were divided by the spectrum of 20 µg mL^{-1} of its degradation product to obtain the ratio spectra. The spectra were transferred to Matlab (R2013b) for signal processing and analysis where; the first derivative of the obtained ratio spectra was employed according to the SGF method through the use of 7-point window size and a cubic model filter. The amplitudes of the first derivative of the ratio spectra that calculated by SGF were measured at 283 nm and were plotted against corresponding concentrations in µg mL^{-1} to build the calibration curve and regression equation was derived.

3.1.3. Multivariate spectrophotometric determination of cefoxitin-sodium in presence of its alkali-induced degradation product using continuous wavelet transform of ratio spectra method [25, 26-29]:

The ratio spectra obtained as under (**3.1.2.)** were transferred to Matlab (R2013b) for signal processing and analysis where the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) =32]. The amplitudes of the transformed signals at 264 nm were measured. The calibration curve was constructed by plotting the amplitudes of the transformed signals at 264 nm versus the corresponding concentrations in µg mL^{-1} and the regression equation was derived.

3.1.4. Multivariate spectrophotometric determination of cefoxitin-sodium in presence of its alkali-induced degradation product using wavelet transform of the first derivative of ratio spectra method [25, 30-34]:

The ratio spectra obtained as under (**3.1.2.)**, then the first derivative of the ratio spectra using $\Delta\lambda$ = 8 nm and a scaling factor of 30 was calculated, The spectra were transferred to Matlab (R2013b) where the first derivatives of ratio spectra were transferred to the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) =32]. The amplitudes of the transformed signals at 229.6 nm were measured and plotted against corresponding concentrations to build the calibration curve.

4.2. **Application to laboratory prepared mixtures**

Different aliquots of cefoxitin-sodium together with its alkali-induced degradation product were transferred from their working solutions into a series of 10-mL volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with water. The spectra of the prepared series from 200 to 400 nm were recorded and stored. The general procedure was followed for each method and the concentrations of cefoxitin-sodium were calculated for each proposed method from the corresponding regression equation.

4.3. Application to pharmaceutical preparation

A stock solution containing (1mg mL^{-1}) cefoxitin-sodium was prepared by dissolving an accurately weighed quantity of well-mixed powder from three vials of Primafoxin® equivalent to 100 mg of cefoxitin-sodium in 60 mL water by shaking for about 10 min. The volume was adjusted to 100-mL with water, a solution containing (0.1 mg mL^{-1}) was obtained by suitable dilution, then analyzed by the proposed methods using aliquots covering the working concentration range and the standard addition technique was applied.

RESULTS AND DISCUSSION

The aim of this work was to develop simple, sensitive and accurate analytical methods for analysis of cefoxitin-sodium in presence of its degradation product. These methods showing advantages over the

reported derivative method in sensitivity with lowest LOD and LOQ values and regression equation showing a marked decrease in the intercept.

Spectral characteristics:

The overlap shown in (Figure 2) prevents direct spectrophotometric determination of cefoxitin-sodium in presence of its degradation product; that overlap eliminated by CCSM, SGF, CWT or DWT.

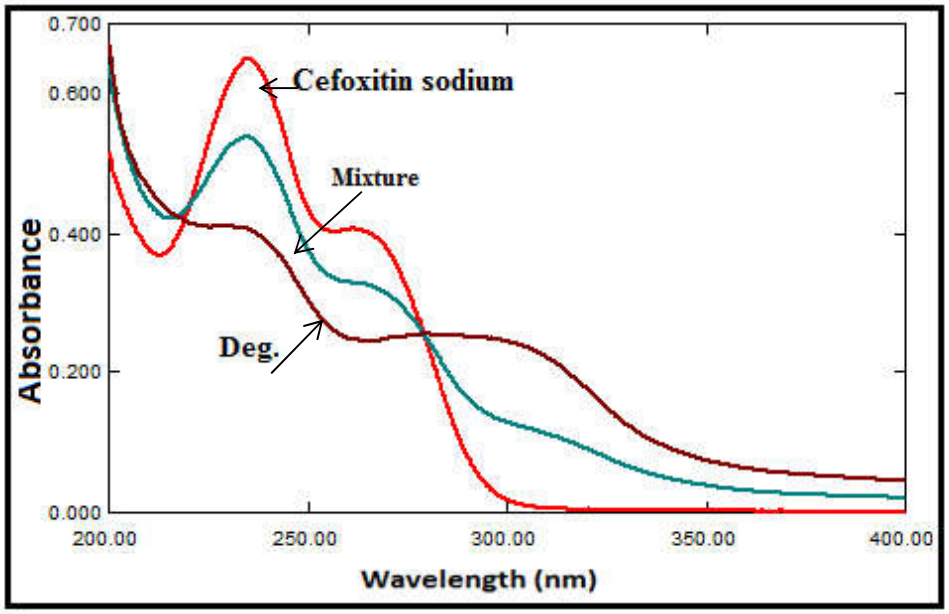

Figure (2) Overlain absorption spectra of cefoxitin-sodium (20 µg mL^{-1}), its alkali-induced degradation product (20 µg mL^{-1}) and a (1:1) mixture containing 10 µg mL^{-1} of each using water as a solvent.

Constant Center (CCSM) Method

In this method, the absorption spectra of degradation product were scanned (**Figure 3)**, and divided by the absorption spectrum of (20 µg mL^{-1}) of cefoxitin-sodium, as shown in (**Figure 4)**, the ratio spectra obtained represent: $\left(\frac{\textbf{Dgradation-product}}{\textbf{Cefoxitin-sodium divisor}} + \textbf{Constant}\right)$

Ratio difference at the two selected wavelengths was represented by:

$$\left(\frac{\textbf{Dgradation-product}}{\textbf{Cefoxitin-sodium divisor}}\right) \textbf{ at } \lambda_{275} - \left(\frac{\textbf{Dgradation-product}}{\textbf{Cefoxitin-sodium divisor}}\right) \textbf{ at } \lambda_{238}$$

The linear relation between the difference of the ratio spectra (at 275 and 238 nm) and the amplitude of the ratio spectra at 275 nm over a range (2-32 µg mL^{-1}) were obtained, the regression equation was computed, and was represented by:

$$\textbf{P}_1\textbf{-P}_2 = \textbf{Slope P}_1 \pm \textbf{intercept} \qquad \textbf{Eq. ……..(1)}$$

Where;

$\mathbf{P_1}$: is the amplitude of the ratio spectra at λ_{275} nm.

$\mathbf{P_2}$: is the amplitude of the ratio spectra at λ_{238} nm.

The amplitude of the ratio spectra of the mixture was recorded at 275 and 238 nm and substituted in equation (1) to obtain the postulated value ($P_{postulated}$).

Where; $P_{postulated}$ is the amplitude corresponding to $\left(\frac{\textbf{Dgradation-product}}{\textbf{Cefoxitin-sodium divisor}}\right) \textbf{ at } \lambda_{275}$

$$\textbf{P}_{275}\textbf{-P}_{238}\textbf{= 0.2383 (degradation-product / cefoxitin divisor) + 0.001} \qquad (r^2 = 1)$$

The constant value (C.V) representing the amplitude corresponding to $\left(\frac{\textbf{Cefoxitin sodium}}{\textbf{Cefoxitin divisor}}\right)$ at λ_{275} nm and was obtained by subtracting the $p_{postulated}$ value from the recorded amplitude ($P_{recorded}$) of the ratio spectra of the laboratory-prepared mixture at λ_{275} nm.

$$\textbf{C.V= P}_{\textbf{recorded}} \textbf{ -P}_{\textbf{postulated}} \text{ at } \lambda_{275} \text{ nm} \qquad \textbf{Eq. ……..(2)}$$

Then original spectra of cefoxitin-sodium in the mixture were obtained by multiplying the obtained *constant value* of the laboratory mixture by the spectrum of the cefoxitin-sodium divisor, as shown in (**Figure 5)**, and this was the most important advantage of this method that; the zero order absorption spectra of the target drug recovered using simple mathematical calculations, CCSM not affected by background noise in lower concentration, the constant value was obtained from subtraction of ($P_{recorded}$ -

$P_{postulated}$), not through plateau, while $P_{postulated}$ was obtained from a regression equation. The concentration of cefoxitin-sodium was determined from the regression equation relating the absorbance of the zero order spectra of cefoxitin-sodium at 235 nm to the corresponding concentrations.

Optimization of experimental conditions: constant center spectrophotometric method involved two complementary steps; the first was the choice of the divisor, the selected divisor should compromise between minimal noise and maximum sensitivity. The divisor concentrations of 20 µg mL^{-1} gave the best results. The second step was the choice of the two wavelengths at which the component of interest shows a significant difference in these two ratio values with concentrations while the ratio spectrum of degradation product shows the same value. The selected wavelengths were 238 and 275 nm ($\Delta P_{238-275}$) gave the best results.

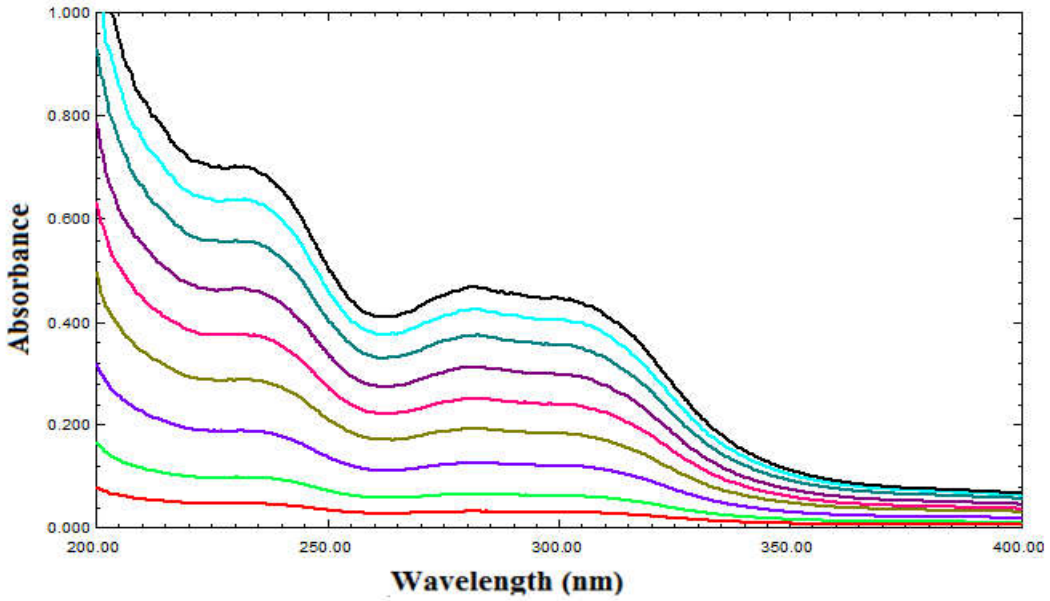

Figure (3): Absorption spectra of cefoxitin-sodium alkali-induced degradation product at various concentrations (2-32 µg mL^{-1})

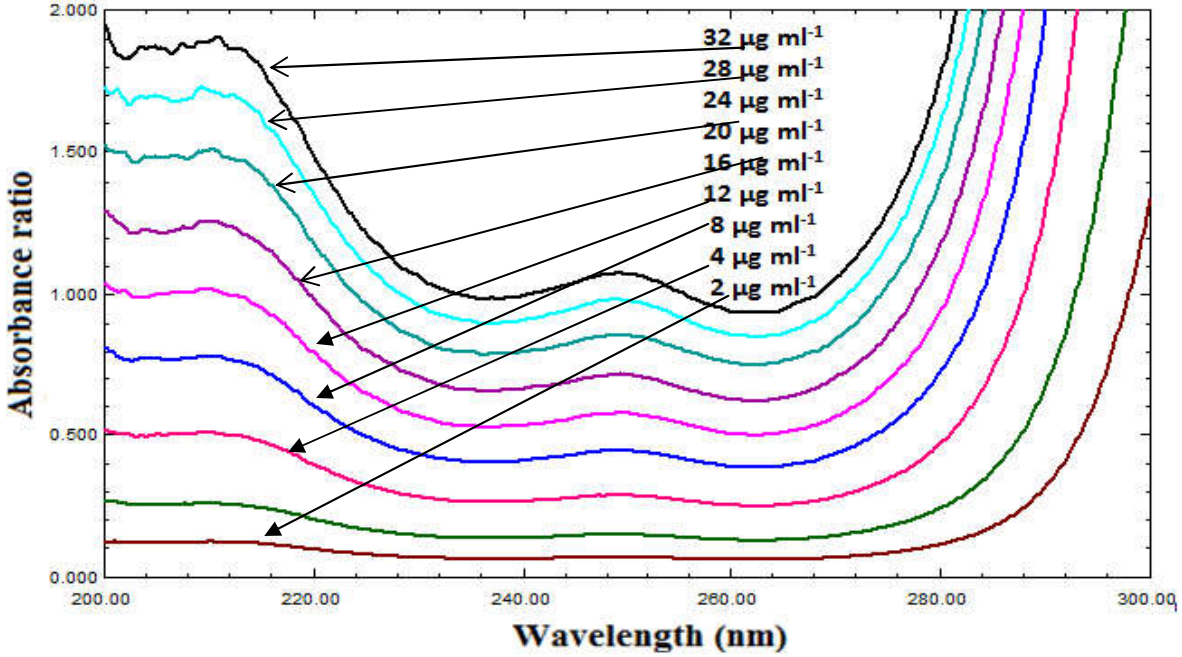

Figure (4): Ratio spectra of cefoxitin-degradation product (2-32 µg mL^{-1}) using (20 µg mL^{-1}) of cefoxitin-sodium as a divisor.

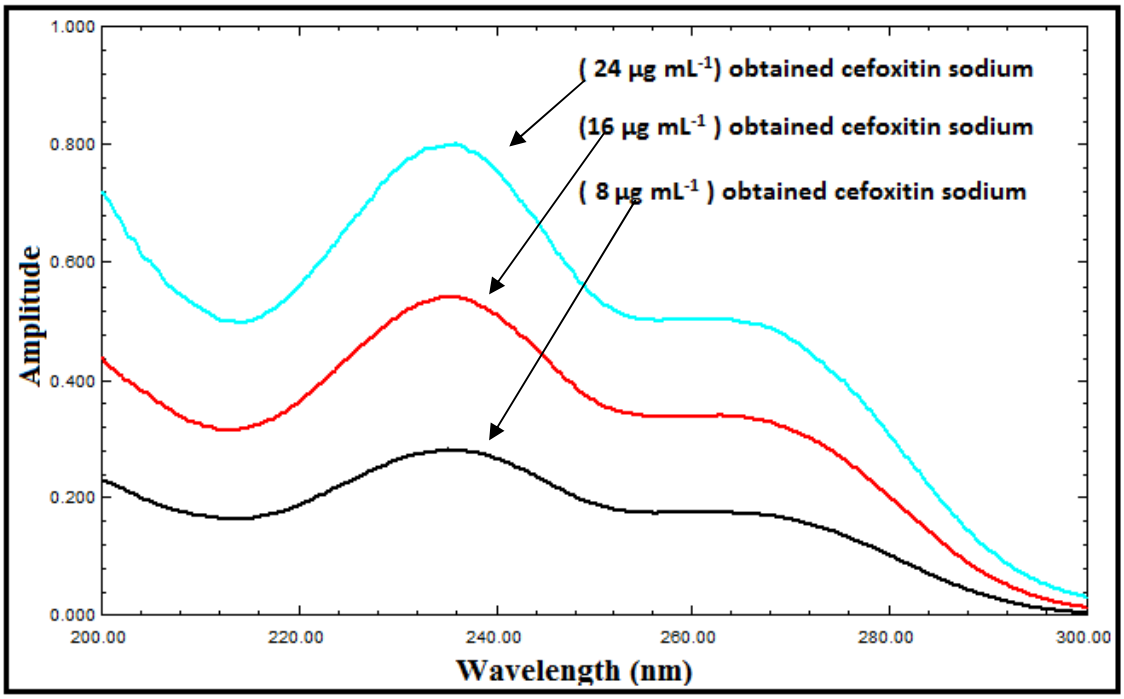

Figure (5): The final spectra of cefoxitin-sodium at various concentrations (2- 32 µg mL^{-1}) after multiplication by the spectrum of 20 µg mL^{-1} of cefoxitin-sodium.

Ratio derivative by Savitsky-Golay filters (SGF) Method

In this method, the ratio spectra of cefoxitin-sodium were obtained using absorption spectrum of its alkali-induced degradation product (20 µg mL^{-1}) as a divisor, (**Figure 6)**. The first derivative of the obtained ratio spectra was employed according to the SGF method by the use of 7-point window size and a cubic model filter. The amplitude of the first derivative of the ratio spectra was calculated by SGF at 283 nm, which proportional to the concentrations of the drug without interference from the divisor, (**Figure 7)**.

Optimization of experimental conditions: different parameters related to the calculation of the Savitsky-Golay coefficients were optimized including the selection of the devisor concentration, function order and the number of points (window size), it was found that, the divisor of concentration 20 µg mL^{-1} was suitable to obtain the ratio spectra and then the first order derivative was applied using 7-point window size and a cubic model filter for processing the signals of the ratio spectra as they give best results.

Continuous wavelet (CWT) transform of ratio spectra method

In this method, the obtained ratio spectra (as described under 5.2.) were employed using *bior 1.1* family with [scale value (a) =32] to get the wavelet coefficients. The amplitudes of these coefficients calculated by CWT at 264 nm were proportional to the concentrations of the cited drug without interference from degradation product, (**Figures 6, 8)**.

Optimization of experimental conditions: different parameters associated with the calculation of the continuous wavelet transform were optimized, included the selection of the divisor concentration, wavelet type and the scaling value, the divisor concentration (20 µg mL^{-1}) was applied to obtain the ratio spectra and *bior 1.1* family with [scale value (a) =32] were applied for their calculations. The transformed signals were measured at 264 nm.

Wavelet transform of the first derivative of ratio spectra (DWT) method

In this method, the ratio spectra obtained (as described under 5.2.) (Figure 6), then the first derivative of the ratio spectra using $\Delta\lambda$ = 8 nm and a scaling factor of 30 was calculated, (Figure 9). In an effort to enhance the sensitivity of the analysis, the first derivatives of ratio spectra were transferred to the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) =32]. The amplitudes of the transformed signals at 229.6 nm were measured, (Figure 10).

Optimization of experimental conditions: The combination of derivative and wavelet transform was performed in an effort to increase the number of zero-crossing points as well as to obtain a higher sensitivity and selectivity as compared to the original derivative or wavelet spectra, different **scaling**

factors and different **smoothing factors** (**Δλ**) values were tested also, different parameters associated with the calculation of the continuous wavelet transform were optimized including; the selection of the divisor concentration, wavelet type and the scaling value, the divisor concentration (20 µg mL^{-1}) was applied to obtain the ratio spectra, then first derivative of the ratio spectra using Δλ = 8 nm and a scaling factor of 30 was calculated, and *bior 1.1* family with [scale value (a) =32] were applied for their calculations, the amplitudes of the transformed signals at 229.6 nm were measured.

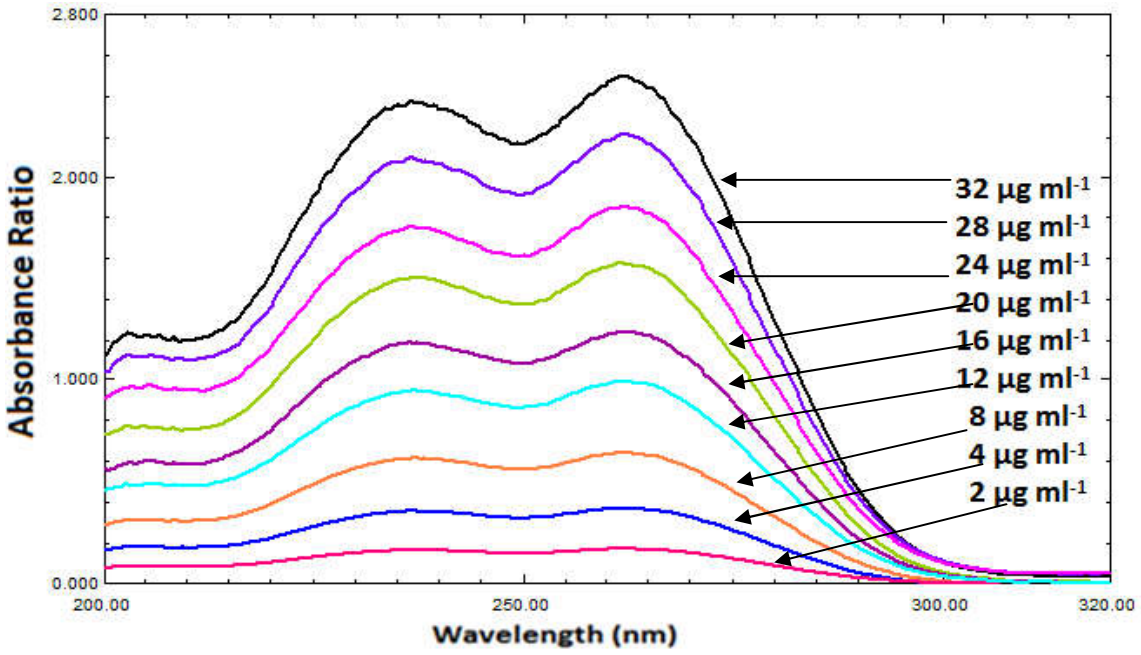

Figure (6): Ratio spectra of cefoxitin-sodium (2-32 µg mL^{-1}) using (20 µg mL^{-1}) of a cefoxitin-degradation product as a divisor.

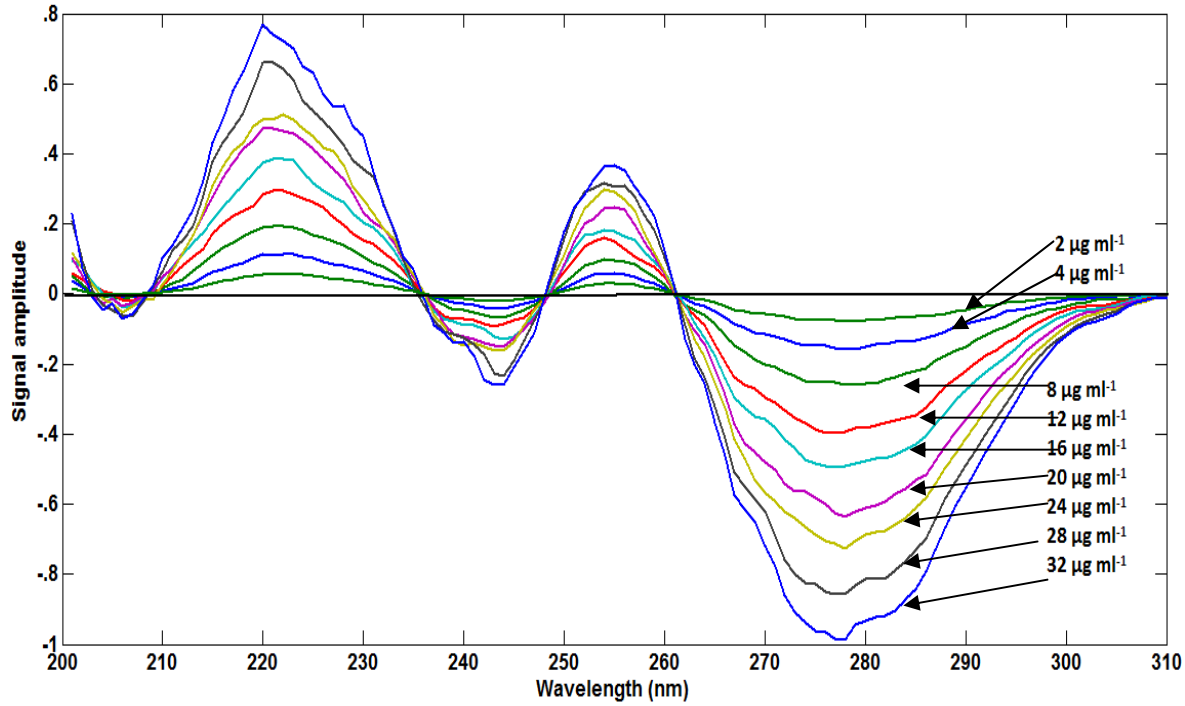

Figure (7): Savitzky-Golay application on the ratio spectra of cefoxitin-sodium (2-32 µg mL^{-1}) using (20 µg mL^{-1}) of its degradation product as a divisor.

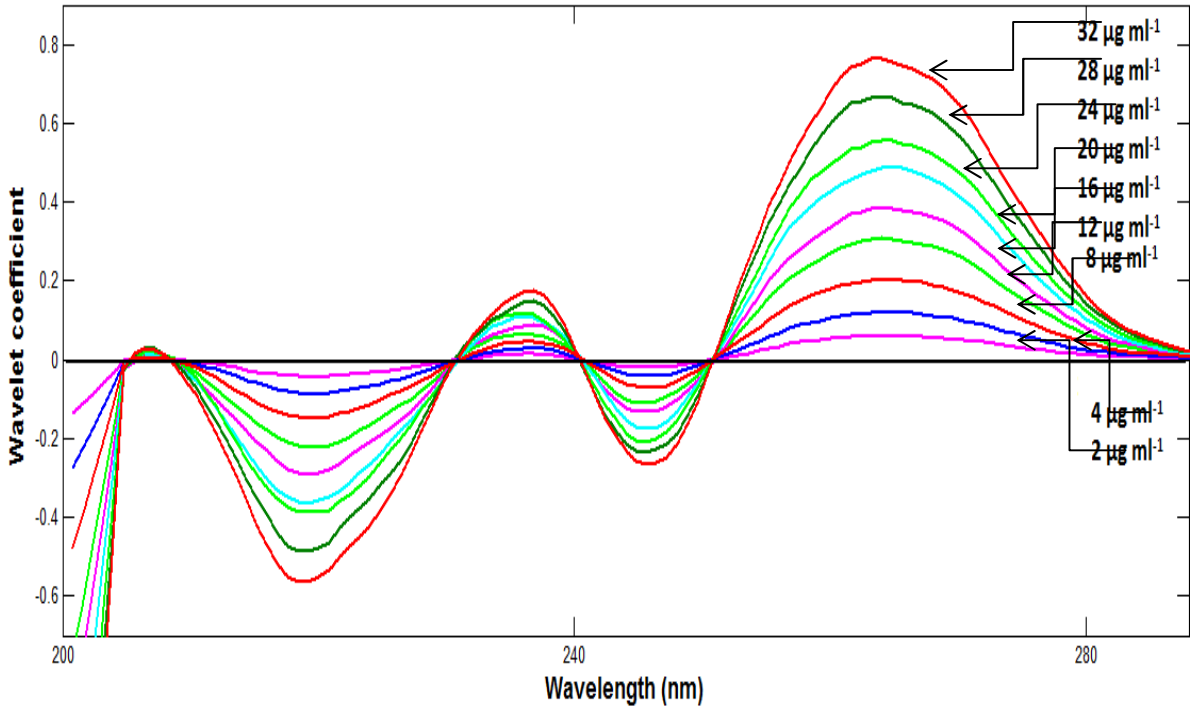

Figure (8): CWT of the ratio spectra of cefoxitin-sodium at various concentrations (2-32 µg mL^{-1}) using 20 µg mL^{-1} of its degradation product as a divisor.

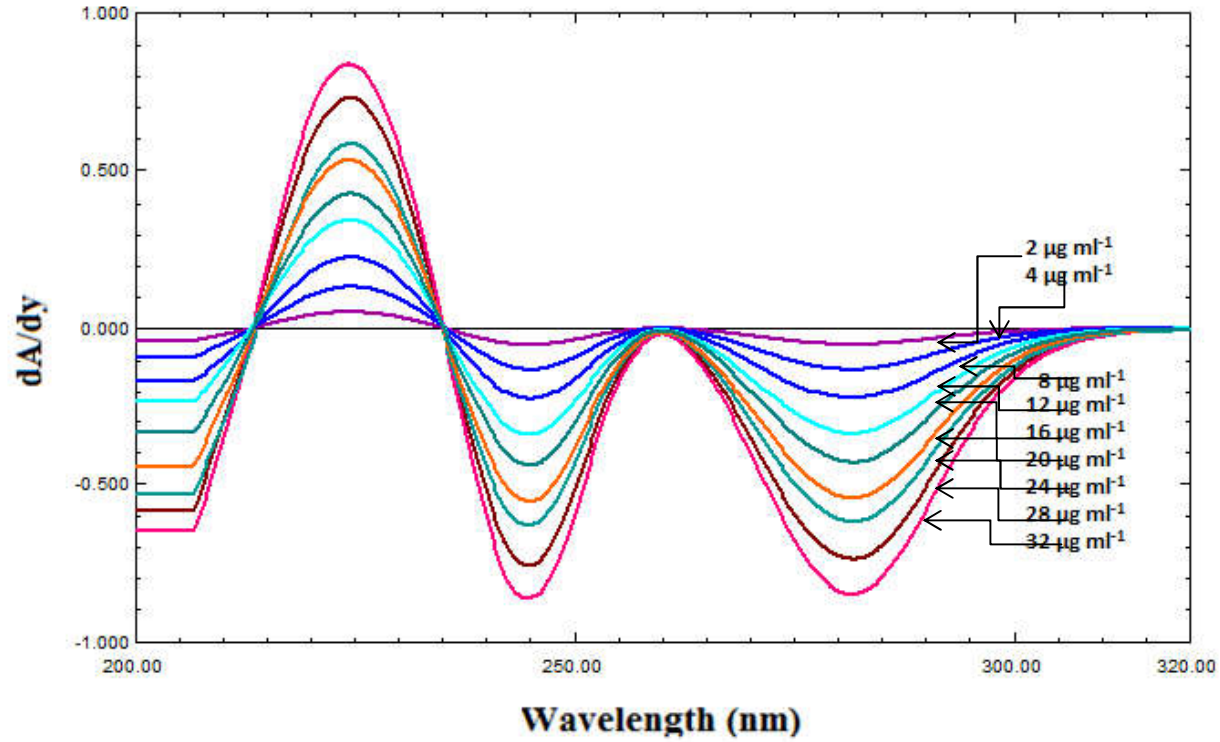

Figure (9): First-derivative spectra of cefoxitin-sodium using (20 µg mL^{-1}) of its degradation product as a divisor.

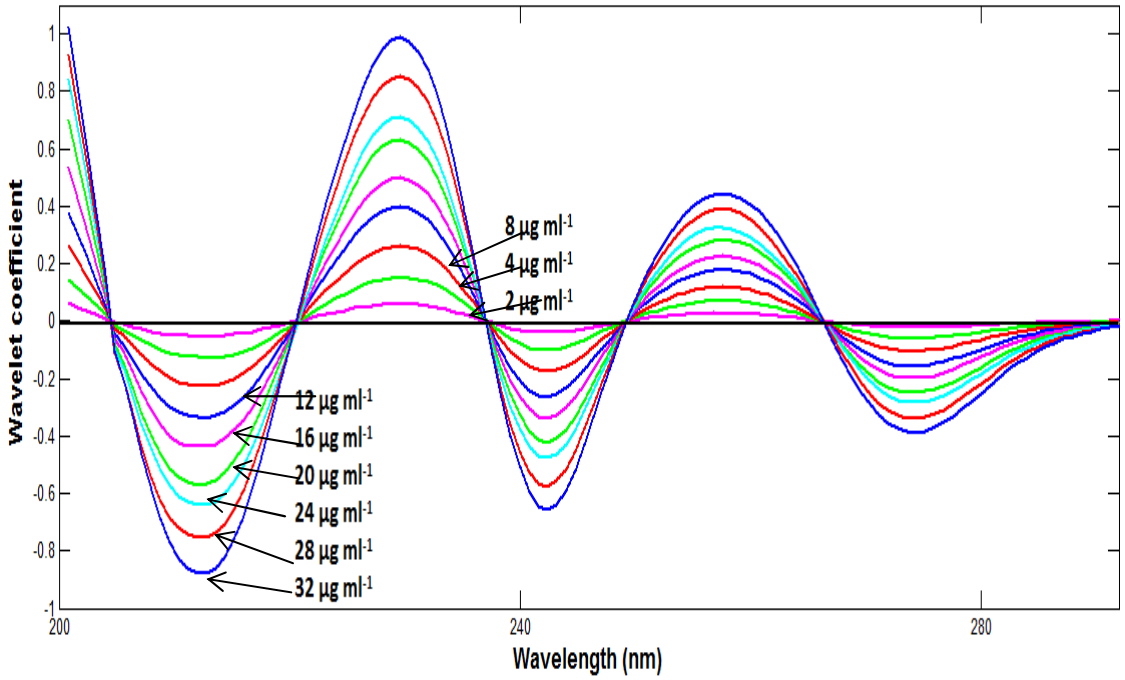

Figure (10): Wavelet transform of the first derivative of ratio spectra for cefoxitin-sodium at various concentrations (2-32 µg mL^{-1}) using 20 µg mL^{-1} of its degradation product as a divisor.

Methods validation [35]

All methods were validated as per ICH guidelines.

- **Linearity:** All methods obey Beer's law in the range of (2-32 µg mL^{-1}). Regression and validation data for the determination of cefoxitin-sodium by the proposed methods were listed in Table (**1**).

Table (1): Regression and validation data for the determination of cefoxitin-sodium by the proposed methods

Parameters	**CCSM**	**SGF**	**CWT**	**DWT**
Accuracy (mean ± SD)[a]	99.59 ± 1.413	100.09 ± 0.990	100.33 ± 1.449	100.99 ± 0.752
Precision				
Repeatability (RSD)[b]	0.879	0.172	0.135	0.034
Intermediate precision(RSD)[c]	1.211	0.198	0.157	0.116
Linearity range	2-32 µg mL^{-1}			
Wavelength(nm)	235	283	264	229.6
Slope ± S.D	0.033 ± 0.018	0.0028 ± 0.0006	0.0305 ± 0.002	0.0234 ± 0.003
Intercept ± S.D	0.0224 ± 0.002	0.0017 ± 0.0003	0.0042 ± 0.005	0.0125 ± 0.001
LOD	0.182	0.321	0.492	0.128
LOQ	0.606	1.071	1.639	0.427
Determination coefficient(r^2)	0.9998	0.9999	0.9998	0.9999

[a]Average of three determinations for three concentrations (4,16 and 28 µg mL^{-1}) repeated three times.
[b]The intraday (n = 3), average of three concentrations (4,16 and 28 µg mL^{-1}) repeated three times within the same day.
[c]The interday (n = 3), average of three concentrations (4,16 and 28 µg mL^{-1}) repeated three times in three days.

Limits of detection and quantitation: LOD was found to be 0.091, 0.321, 0.492 and 0.128 µg mL^{-1}, while LOQ was found to be 0.303, 1.071, 1.639 and 0.427 µg mL^{-1} for CCSM, SGF, CWT and DWT method, respectively, **table (1).**

Accuracy and precision: Accuracy and precision were tested and the accuracy was represented as percentage recovery **(%R)**, while precision was represented as the percentage relative standard deviation **(% RSD)**, **table (1).**

Specificity: The specificity of the proposed methods was confirmed by analysis of laboratory mixtures of cefoxitin-sodium with its alkali-induced degradation product. All the proposed methods able to detect the cited drug in presence of up to 81.25 % of its degradation product, the results were listed in the table (**2**).

Table (2): Determination of intact cefoxitin-sodium in laboratory prepared mixtures with its alkali-induced degradation product by the proposed methods.

Conc. of cefoxitin-sodium (µg mL^{-1})	Deg. (µg mL^{-1})	%R			
		CCSM*	SGF*	CWT*	DWT*
6	26	101.67	101.17	99.67	101.17
8	24	100.38	100.38	99.00	101.38
16	16	101.25	100.75	99.13	100.81
24	8	100.83	100.75	99.71	99.91
26	6	100.88	101.19	99.65	99.50
Mean ± SD%		**101.00 ± 0.485**	**100. 85 ± 0.339**	**99.43 ± 0.339**	**100.55 ± 0.814**

** Average of three determinations.*

- **Recovery study by standard addition technique:** Validity of the proposed was performed by adopting standard addition technique. Results were listed in the **table (3).**

Table (3): Application of standard addition technique to the analysis of **Primafoxin®** vials by applying the proposed methods.

Pharmaceutical taken, equivalent to cefoxitin-sodium (10 µg ml^{-1})	Added standard (µg mL^{-1})	Constant Center*	SGF*	CWT*	DWT*
Primafoxin® vials Batch No.(109)	6	100.78	100.83	100.55	99.60
	12	101.46	99.70	99.84	98.88
	20	98.85	100.92	101.21	99.77
Mean ± RSD%		100.36 ±1.349	100.48 ±0.676	100.53 ± 0.681	99.42 ± 0.475

**Average of three determination.*

Statistical analysis

Statistical comparison of the results obtained by applying the proposed methods and those obtained by applying the reported method [17] showed less calculated t and F values than the theoretical ones indicating no significant difference between the proposed and the reported methods with respect to accuracy and precision, Table (**4**).

Table (4): Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and reported method for determination of cefoxitin-sodium in Primafoxin® vials

Parameter	CCSM	SGF	CWT	DWT	Reported method[d]
Mean[a]	99.66	100.43	99.89	100.72	99.02
S.D.	1.349	1.235	1.336	1.189	0.915
n[b]	5	5	5	5	5
Variance	1.820	1.525	1.785	1.413	0.837
***t*-test** (2.306)[c]	1.895	1.895	1.895	1.895	——
***F*-value** (6.388)[c]	2.175	1.823	2.134	1.823	——

[a] *The mean of percent recovery of the pharmaceutical preparation.*
[b] *Number of experiments.*
[c] *The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05).*
[d] *Cefoxitin-sodium was stressed with 4.5 M sulphuric acid on boiling water bath for 20 min, then 2nd derivative spectra were recorded at 278 nm*.

CONCLUSION

This work introduced; four, simple, sensitive, selective, accurate, rapid and economical spectrophotometric methods for determination of cefoxitin-sodium in presence of its alkali-induced degradation product without any separation steps. The proposed methods were validated according to ICH guidelines and the good results obtained conforming high sensitivity. The proposed chemometric methods (SGF, CWT, and DWT) have advantages over other chemometric methods as PCR, PLs and CLS methods as it can be performed using simple absorption spectra and no need to build an experimental design. Also, CWT and DWT methods have advantages over CCSM and SGF methods as peak amplification and more zero-crossing points, while the most sensitive method was DWT with lowest LOD and LOQ

values. Thus, the proposed methods valuable for analysis of cefoxitin-sodium in pure form and in its powder for injection in quality control laboratories.

CONFLICT OF INTEREST

There is no conflict of interest.

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